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DOI: https://doi.org/10.1161/ATVBAHA.107.157321

Originally published at:
Riewald, Matthias; Schuepbach, Reto A (2008). Protective signaling pathways of activated protein C in endothelial cells. Arteriosclerosis, Thrombosis, and Vascular Biology, 28(1):1-3.
DOI: https://doi.org/10.1161/ATVBAHA.107.157321

Protective Signaling Pathways of Activated Protein C in Endothelial Cells

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Introduction

Activated protein C (APC) has been reported to improve survival in patients with severe sepsis.[1] Protein C (PC) is physiologically activated on the endothelial cell surface by the key procoagulant enzyme thrombin, and APC downregulates thrombin formation in a negative feedback loop.[2] This anticoagulant effect of APC is unlikely to explain its benefit in systemic inflammation because other anticoagulants did not improve survival in septic patients. How the protective effects of APC in systemic inflammation are mediated has thus received a considerable amount of attention in recent years.

In vitro studies have shown that incubation of cultured endothelial cells with APC leads to a number of potentially antiinflammatory cellular changes. These include downregulation of the expression of adhesion molecules, enhanced barrier function in cell monolayers, and effects on gene expression that result in decreased susceptibility to apoptosis. Most of these cellular responses have been shown to require the binding of APC to endothelial cell protein C receptor (EPCR) and cleavage-mediated activation of protease activated receptor-1 (PAR1).[3–6] EPCR may not only enhance activation of PAR1 by APC but also modify downstream responses through other mechanisms.[7–9] EPCR is a transmembrane receptor with a very short cytoplasmic domain and an extracellular domain that binds both PC and APC. EPCR recruits and positions PC/APC on the cell surface, and EPCR binding enhances activation of PC. PAR1 is the prototypical thrombin receptor and belongs to a family of 7-transmembrane G protein–coupled receptors. Enzymatic cleavage of PAR1 exposes a new extracellular N terminus that acts as a tethered activation ligand. Importantly, in mouse models of stroke and endotoxemia EPCR-dependent PAR1 signaling is required for protective effects of APC.[5,10] However, it is unknown which cellular effects of APC lead to protection in vivo, and it remains possible that mechanisms independent of EPCR-PAR1 also contribute to protective effects of APC.

In the December 2007 issue of *Arteriosclerosis, Thrombosis, and Vascular Biology*, O'Brien and colleagues[11] report that APC downregulates the expression of tumor necrosis factor (TNF)-related apoptosis inducing ligand (TRAIL) in endothelial cells dependent on the activation of ERK1/2 and the transcription factor EGR-1. TRAIL is best known for its potential to induce apoptosis specifically in cancer cells but has also been implicated in the regulation of inflammatory responses.

EPCR-Independent PAR1 Signaling by APC?

O'Brien et al report that the downregulation of TRAIL by APC required cleavage of PAR1 but was independent of APC binding to EPCR. Surprisingly, the induction of ERK1/2 phosphorylation and EGR-1 activation by APC–PAR1 signaling were also EPCR independent, whereas previous studies reported the same pathways to be dependent on EPCR.[3,12] These divergent results may be explained by the use of different experimental conditions and cell lines. O'Brien et al used specific antibodies blocking the interaction between APC and EPCR and downregulation of EPCR expression by siRNA to establish the role of EPCR in APC-PAR1 signaling. Control experiments demonstrated that effects of APC on the adhesion receptor expression, staurosporine-induced apoptosis, and endothelial barrier integrity were dependent on APC binding to EPCR, which is consistent with previous results. Do these interesting new data establish that there is in fact relevant EPCR independent PAR1 activation by APC (Figure).Studies in purified systems[13] and assays using an APC mutant without the EPCR binding domain[14] have demonstrated that at high concentrations APC can directly cleave PAR1 even if EPCR binding is not available. EPCR facilitates PAR1 cleavage by lower APC concentrations by recruiting and positioning the protease to specific domains on the plasma membrane for efficient PAR1 cleavage.[14] Thus, one possibility is that the EPCR independent downregulation of TRAIL is caused by coreceptor independent activation of PAR1 by APC. The resulting very low rate of PAR1 activation may support TRAIL downregulation because of a high sensitivity of this pathway to PAR1 signaling. In contrast, other downstream pathways such as protection from apoptosis or barrier enhancement may require a higher rate of PAR1 activation. It is also important to keep in mind that antibody blockade of EPCR or siRNA-mediated downregulation are not expected to be complete. It is difficult to rule out that residual availability of EPCR still mediates at least some of the observed effects, especially if higher concentrations of APC are used for prolonged incubation times. These issues could in the future be addressed using EPCR independent activators of PAR1 such as thrombin or variants of APC. APC binds to EPCR through its Gla domain. To ultimately prove that the responses do not require EPCR binding, APC variants with a deleted or mutated Gla domain could be used.[14] Furthermore, experiments using cell lines lacking EPCR could be used to definitely establish the role of EPCR in responses to wild-type APC.

Another possibility put forth by the authors is that a not yet identified coreceptor is involved in the PAR1-dependent downregulation of TRAIL by APC. EPCR colocalizes with PAR1 in lipid rafts, and ligand binding to EPCR may modulate its compartmentalization and affect downstream signaling responses.[7–9,14] A novel coreceptor may also localize APC in specific microdomains where different signaling complexes are assembled and where PAR1-dependent signaling specifically and efficiently downregulates TRAIL expression. The identification of such novel cofactor for APC-PAR1 signaling will be required to test this model in future studies.

Role of the Sphingosine-1 Phosphate Pathway in Mediating APC Effects

Sphingosine 1-phosphate (S1P) is a biologically active lipid that is generated by cellular sphingosine kinases (SK) and S1P signaling is mediated by the S1P receptor family of seven-transmembrane G-protein-coupled receptors. S1P can induce responses in endothelial cells that resemble APC-mediated responses, including enhanced barrier function, antiapoptotic effects, and downregulation of adhesion molecules.[15–17] Indeed, EPCR-dependent protective effects of APC on the barrier integrity of an endothelial cell monolayer have been shown previously to require SK activity and expression of the S1P receptor-1 ($S1P_1$).[4,8] The current study by O'Brien et al shows that SK and $S1P_1$ are required for the downregulation of TRAIL by APC. These novel data implicate crossactivation of the S1P pathway in potentially antiapoptotic effects of APC signaling for the first time.

It will be interesting to establish the role of S1P signaling in other responses to APC, including protective effects on staurosporine-induced apoptosis, adhesion molecule expression, and most importantly in beneficial effects of APC in models of systemic inflammation. Infusion of S1P has been shown to be protective in models of endotoxin-induced acute lung injury[16] and it is possible that protective in vivo effects of APC require crossactivation of this pathway, including the activation of endothelial cell $S1P_1$. Even if S1P pathway crossactivation is indeed a general requirement for responses to APC in tissue culture, S1P receptor agonists and APC signaling will target different cell populations in vivo. This is because of the fact that in vivo the PC pathway depends on expression of cellular cofactors such as EPCR and thrombomodulin. The relative specificity of PC pathway signaling for endothelial cells may avoid detrimental side effects of S1P receptor activation in other cell types, eg, direct effects on lymphocyte migration, in the treatment of inflammatory conditions.

Clearly, very little is known with regard to the mechanism of S1P receptor crossactivation by APC. How exactly do SK and $S1P_1$ contribute to the signaling? Given that plasma contains large amounts of S1P, it is difficult to explain how the S1P pathway can be relevant for APC signaling. Perhaps autocrine $S1P_1$-dependent signaling of endothelial cell-produced and locally secreted S1P is more efficient compared with plasma S1P, which is expected to be largely bound to plasma proteins. Alternatively, APC has been shown to induce colocalization of EPCR with $S1P_1$, and $S1P_1$ may be activated through other mechanisms that do not necessarily involve S1P binding, eg, cross-phosphorylation events.[8]

In conclusion, the new results identify TRAIL downregulation as a novel APC-mediated response and they highlight that novel receptors and signaling pathways may be involved in protective APC signaling in endothelial cells. A better mechanistic understanding of how cells sense the proteolytic activity of APC in their microenvironment and how they respond may eventually lead to novel approaches to treat patients with sepsis and other disorders where the inflammatory response plays a key role, including myocardial infarction and stroke.

References

1. GR, Vincent JL, Laterre PF, LaRosa SP, Dhainaut JF, Lopez-Rodriguez A, Steingrub JS, Garber GE, Helterbrand JD, Ely EW, Fisher CJ. Efficacy and safety of recombinant human activated protein C for severe sepsis. N Engl J Med. 2001; 344: 699–709.

2. Esmon CT. The protein C pathway. Chest. 2003; 124: 26S–32S.
3. Riewald M, Petrovan RJ, Donner A, Mueller BM, Ruf W. Activation of endothelial cell protease activated receptor 1 by the protein C pathway. Science. 2002; 296: 1880–1882.
4. Feistritzer C, Riewald M. Endothelial barrier protection by activated protein C through PAR1-dependent sphingosine 1-phosphate receptor-1 crossactivation. Blood. 2005; 105: 3178–3184.
5. Mosnier LO, Zlokovic BV, Griffin JH. The cytoprotective protein C pathway. Blood. 2006.
6. Bae JS, Yang L, Manithody C, Rezaie AR. Engineering a disulfide bond to stabilize the calcium-binding loop of activated protein C eliminates its anticoagulant but not its protective signaling properties. J Biol Chem. 2007; 282: 9251–9259.
7. Esmon CT, Xu J, Gu JM, Qu D, Laszik Z, Ferrell G, Stearns-Kurosawa DJ, Kurosawa S, Taylor FB Jr, Esmon NL. Endothelial protein C receptor. Thromb Haemost. 1999; 82: 251–258.
8. Finigan JH, Dudek SM, Singleton PA, Chiang ET, Jacobson JR, Camp SM, Ye SQ, Garcia JG. Activated protein C mediates novel lung endothelial barrier enhancement: role of sphingosine 1-phosphate receptor transactivation. J Biol Chem. 2005; 280: 17286–17293.
9. Bae JS, Yang L, Manithody C, Rezaie AR. The ligand occupancy of endothelial protein C receptor switches the PAR-1-dependent signaling specificity of thrombin from a permeability-enhancing to a barrier-protective response in endothelial cells. Blood. In press.
10. Kerschen EJ, Fernandez JA, Cooley BC, Yang XV, Sood R, Mosnier LO, Castellino FJ, Mackman N, Griffin JH, Weiler H. Endotoxemia and sepsis mortality reduction by non-anticoagulant activated protein C. J Exp Med. 2007; 204: 2439–2448.
11. O'Brien LA, Richardson MA, Mehrbod SF, Berg DT, Gerlitz B, Gupta A, Grinnell BW. Activated protein C decreases tumor necrosis factor related apoptosis-inducing ligand by an EPCR independent mechanism involving Egr-1/Erk-1/2 activation. Arterioscler Thromb Vasc Biol. 2007; 27: 2634–2641.
12. Uchiba M, Okajima K, Oike Y, Ito Y, Fukudome K, Isobe H, Suda T. Activated protein C induces endothelial cell proliferation by mitogen-activated protein kinase activation in vitro and angiogenesis in vivo. Circ Res. 2004; 95: 34–41.
13. Kuliopulos A, Covic L, Seeley SK, Sheridan PJ, Helin J, Costello CE. Plasmin desensitization of the PAR1 thrombin receptor: kinetics, sites of truncation, and implications for thrombolytic therapy. Biochemistry. 1999; 38: 4572–4585.
14. Bae JS, Yang L, Rezaie AR. Receptors of the protein C activation and activated protein C signaling pathways are colocalized in lipid rafts of endothelial cells. Proc Natl Acad Sci. U S A. 2007; 104: 2867–2872.
15. Lee MJ, Thangada S, Claffey KP, Ancellin N, Liu CH, Kluk M, Volpi M, Sha'afi RI, Hla T. Vascular endothelial cell adherens junction assembly and morphogenesis induced by sphingosine-1-phosphate. Cell. 1999; 99: 301–312.
16. McVerry BJ, Garcia JG. In vitro and in vivo modulation of vascular barrier integrity by sphingosine 1-phosphate: mechanistic insights. Cell Signal. 2005; 17: 131–139.
17. Whetzel AM, Bolick DT, Srinivasan S, Macdonald TL, Morris MA, Ley K, Hedrick CC. Sphingosine-1 phosphate prevents monocyte/endothelial interactions in type 1 diabetic NOD mice through activation of the S1P1 receptor. Circ Res. 2006; 99: 731–739.

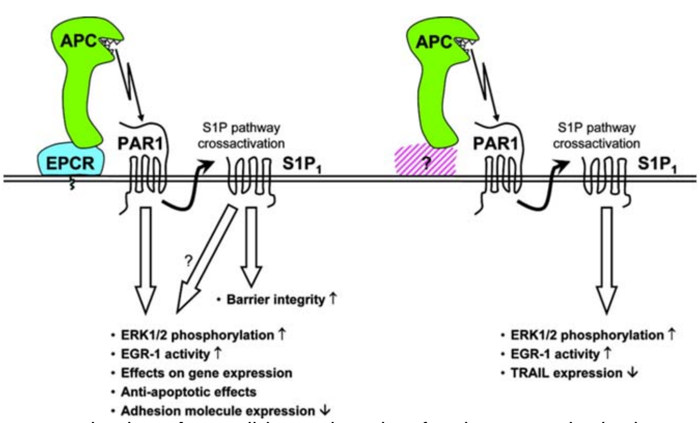

Figure. Proposed Signaling Pathways for APC. APC is endothelial cell surface-recruited and -positioned by binding to EPCR, and the EPCR-bound APC can efficiently cleave and activate PAR1. At least some of the protective responses downstream from PAR1 require the activity of cellular sphingosine kinases and expression of the sphingosine 1-phosphate receptor-1 (S1P1). In this issue of Arteriosclerosis, Thrombosis, and Vascular Biology, O'Brien et al11 report that APC decreases expression of the TNF family member TRAIL independent of EPCR binding but dependent on PAR1 and S1P pathway crossactivation. A possible explanation for these results is that a coreceptor different from EPCR mediates efficient cleavage and activation of PAR1 by APC and channels downstream effects toward downregulation of TRAIL. Another possibility is that downregulation of TRAIL may be a very sensitive readout for APC-PAR1 signaling. The downstream response is obtained even when PAR1 activation is comparatively inefficient in the absence of EPCR binding, whereas more robust PAR1 activation is required to enable signaling pathways mediating other antiinflammatory cellular changes.